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Anaerobic Co-digestion of Giant Reed for Biogas Recovery

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ABSTRACT

This study investigated the feasibility of anaerobic co-digestion of giant reed (GR) inoculated with waste manure as a co-substrate for biogas production. The performance of co-digestion was evaluated in 4 anaerobic digesters operated in batch mode at different conditions. The effects of alkali pretreatment with NaOH (4% w/v) solution, inoculum type, and thermal condition were studied. The results demonstrated that the alkali-pretreatment of GR enhanced the biogas generation by about 15% at mesophilic conditions. Thermophilic conditions enhanced the biogas recovery from both alkali-free and alkali pretreated GR by 15% and 127%, respectively. The kinetic study of the co-digestion process of GR for biogas recovery suggested a significant agreement between measured and predicted values obtained by ***Modified Gompertz Model*** with correlation coefficients ≥ 0.98 indicating favorable conditions for the co-digestion of inoculated GR.

Key words: giant reed, biogas, digestion, alkali pretreatment, methane, chicken dung

الهضم المشترك اللاهوائي للقصب البري لاستخلاص الغاز الحيوي

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الخلاصة

تهدف هذه الدراسة الى اختبار امكانية تطبيق الهضم المشترك اللاهوائي للقصب البري مع فضلات الحيوان لإنتاج الغاز الحيوي. تم تقييم أداء الهضم المشترك وذلك بتشغيل سبعة مفاعلات هضم لاهوائيٍ تعمل جميعها بنمطِ التشغيل الدفعي بظروف تشغيلية مختلفة وتم دراسة تأثير العوامل التالية : المعالجة الكيمياوية التمهيدية للقصب بأستخدام محلول هيدروكسيد الصوديوم بتركيز (4% w/v) ، نوع المادة المضافة كمصدر للبكتريا (تحديدا مخلفات الماشية والدجاج)، و درجة الحرارة. أظهرت النتائج زيادة واضحة في انتاجية الغاز الحيوي من القصب المعالج بالقاعدة مقارنة بكميته المتحررة من القصب الغير معالج وذلك بنسبة 15% عند ظروف حرارة معتدلة.. أظهرت النتائج أيضا زيادة في كمية الغاز الحيوي المتحرر تحت ظروف الحرارة المرتفعة مقارنة بظروف الحرارة المعتدلة للقصب الغير معالج والمعالج بالقاعدة بنسبة زيادة حوالي 15%و 127% ،على التوالي. لغرض وصف عملية الهضم اللاهوائي المشترك، تم تطبيق الموديل الرياضي الخاص لهذه التطبيقات وهو ***Modified Gompertz Model*** وقد اظهرت نتائج تطابقا كبيرا بين نتائج القياسات المختبرية وتلك التي تم احتسابها من الموديل الرياضي وبمعامل ارتباط ≥ 0.98 للظروف التي تم تطبيقها لعملية الهضم اللاهوائي المشترك للقصب.

الكلمات الرئيسية: القصب البري، الغاز الحيوي، الهضم، المعالجة الاولية القاعدية، الميثان، فضلات الدجاج.

1. INTRODUCTION

The ease of access to the fossil fuels during almost two centuries had decreased the available fossil fuel reservoirs, resulting in prices raising, therefore, the energy supply has become one of the most important global problems, **Deublein**, and **Steinhauser, 2008**. The application of biotechnology to produce commodity products such as, fuels, chemicals, and materials offering benefits regarding to sustainable resource supply and environmental quality is an emergent area of intellectual endeavor and industrial practice with great promise, **Lynd, et al., 1999**. Lignocellulose is a material usually exists as the primary components of different wastes disposed from various industries, forestry, agriculture and municipalities. Cellulose is the most abundantly available organic molecule on Earth, is mainly found as a structural component of plant and algal cell walls, and is produced by some animals, such as tunicates, and several bacteria, **Lynd, et al., 2002**. Cellulose chains in primary plant cell walls have degree of polymerization in the range from 5000 to 7500 glucose monomer units, 10000 for wood, and 15000 for cotton **O'Sullivan, 1997**. Hydrolysis of this material is the first step for conversion by anaerobic digestion to biogas (methane). However, enzymatic hydrolysis of lignocelluloses without pretreatment is usually not so effective because of high stability of the materials to enzymatic or bacterial attacks. Pretreatment helps to enhance the process of hydrolysis. Conversion of plant cellulose into ethanol has been industrially achievable since the late Nineteenth Century. However, the near insolubility of cellulose in aqueous solvents initially required physical separation of pure cellulose from plant material and harsh acid hydrolysis to produce the glucose used in fermentation, **O'Dell, et al., 2012**. Fungi and bacteria possess enzymes of laccases, hemicellulases and cellulases, which efficiently degrade lignin, hemicellulose and cellulose, respectively **Baldrian** and **Valášková, 2008**. It is known that excess sludge with low organic content always lead to failure the anaerobic digestion, so the following research of, **Yan, et al., 2013,** gave a solution for this problem by employing mild thermal pretreatment at thermal conditions between (50-120) ◦ C, which has drawn much attention due to less energy consumption and no chemical addition. Where the experimental results showed a gradually rising of soluble organic matter concentration with temperature resulting in biochemical methane enhancement 142.6±2.5 ml/g of volatile solids under mild thermal pretreatment 100 ◦ C and digestion time 20 d. **Cheng** and **Zhong, 2014,** observed biogas yield from anaerobic digestion of inoculated cotton stalk (CS) reached 175−180 ml/g VS. Cotton stalk CS was proven to be a promising co-substrate in the digestion with swine manure (SM) as inoculum. CS/SM ratio of 50:50 with a C/N ratio of 25 was found to be the best conditions for biogas yield with increases up to 1.8- and 1.9-fold, respectively compared to the control. The highest biogas yield of 449 ml/g VS was obtained for the co-digestion of SM with CS pretreated by NaOH, which was 241−255% of those achieved with using the control. **Ismail** and **Talib, 2014,** studied the recycling of date palm wastes (DPWs) for biogas production. It was found that biogas yield from inoculated DPWs had exceeded its production from DPWs without inoculation by an increment of 140% at mesophilic conditions. Also, biogas recovery from pretreated DPWs was 52% higher than the yield from untreated DPWs at mesophilic conditions. Thermophilic conditions improved the productivity by 23%. **Sharma, et al., 2016,** illustrated the effect of alkaline and acid pre-treatment on different sizes of wheat straw on biogas quality and quantity. It was concluded that untreated wheat straw gave a biogas production of 104 ml/g VS and methane yield of 64%. It was observed by using 1%, 2%, 5% NaOH concentrations for pretreatment, the biogas production was 124, 128, 126 ml/g VS with methane content of 66%, 69%, 71%, respectively. Whereby, pretreatment of wheat straw with 1%, 2%, 5% acid produced 130, 140 and 134 ml biogas /g VS and methane content of 68%, 72%, 75%, respectively.

However, to our knowledge none of the previously reported studies have dealt with the anaerobic co-digestion of cellulosic giant reed for biogas production. This study aimed to investigate the potential of giant reed co-digestion for biogas production. The effects of pretreatment, type of inoculum, and temperature conditions were studied. The application of ***Modified Gompertz Model*** for kinetic study was also considered.

2. MATERIALS AND METHODS

2.1 Materials

Wild giant reed (GR) is abundantly found in almost everywhere in Iraq and world-wide as well, in particular in wet areas. In this study, GR was collected from Al-Musayyib river bank area. The collected GR stacks were manually cleaned and carefully washed with tab water to remove sand and undesirable particulates. Then after, the cleaned reed stacks and leaves were air dried, and were cut into small pieces, each of approximately 5 cm length ready to be further crushed into smaller size fibrous shaped particles. Chicken dung was used as a rich bacterium source to inoculate the GR. It was collected from the nearest poultry houses, air-dried, crushed to powder size particles, and then stored in a clean tightly closed plastic container. All chemical reagents utilized in this study were of analytical grade as given in **Table 1.**

2.2 Methods of Analysis

2.2.1 Total solids, Volatile solids, pH, and C/N ratio

These tests were performed in triplicate according to the procedures reported in the *standard methods*, **APHA, 1998**. Sample of 25-50 g was dried at 105ºC to drive off the water in the sample. The residues was cooled, weighed to calculate TS, and dried again at 550- 600°C for 4 h to drive off volatile solids in the sample**.** pH was measured using pH meter (Model: WTW, Inolab 720).

Measurements of C/N ratio included carrying out the ***Kjeldahl analysis*** to find the crude protein (CP) and nitrogen (N) contents in GR in three main steps including digestion, distillation, and titration as according to **Bugodo, et al., 2008** and **Abba, et al., 2014** as follows:

Digestion, this step involved the decomposition of nitrogen in the sample using concentrated sulfuric acid (98 %) to produce ammonium sulfate as the reaction end product. This was carried out by adding 0.5 g of the sample in kjeldahl digestion tube with 1 g of the catalyst $CuSO_4.K_2SO_4$ followed by the addition of 15 ml H_2SO_4. The content was then gently heated to 360°C in the Kjeldahl digestion unit until the digest became clear indicating total conversion of nitrogen into ammonia.

Distillation, after the completion of digestion process, the Kjeldahl digestion tubes were cooled and diluted with 25 ml distilled water. The solution turned blue due to the reaction between the catalyst and water. Then each Kjeldahl digestion tube was placed in the crude protein measuring device. A receiving flask containing 50 ml boric acid (1%) to capture the ammonia, and red methyl dye. This red dye turned into green indicating the existence of nitrogen which was released due to the reaction between the contents of Kjeldahl flask and NaOH solution (40 % as aliquot).

Titration with HCl, in order to quantify the amount of ammonia in the receiving flask, a standard solution of HCL was carefully added by pipette until the green color of solution in the conical flask turned to red color.

Calculations of nitrogen, carbon amounts, and C/N
The crude protein (CP), nitrogen, carbon, and C/N were calculated using equations (1), (2), (3), and (4), respectively, **Bugodo, et al., 2008**, and **Abba, et al., 2014**:

$$Protein\ (\%) = \frac{1.401\ x\ M\ x\ 6.25}{g\ of\ sample}\ x\ (ml\ titrant - ml\ blank) \quad (1)$$

Where:
M: the molarity of the acid (0.1 M)

$$Nitrogen\ (\%) = \frac{Protein\ (\%)}{6.25} \quad (2)$$

$$Carbon\ (\%) = 0.58\ x\ organic\ matter \quad (3)$$

$$C{:}N = \frac{\%\ Organic\ carbon\ in\ the\ sample}{\%\ Nitrogen\ in\ the\ sampleof\ sample} \quad (4)$$

The average measured values of total solids (TS), volatile solids (VS), and C/N ratio for the tested samples are given in **Table 2.**

2.2.2 Measurement of produced biogas

In this study, the produced biogas was measured by three different methods as follows:
Manometer, it consisted of U-shaped glass tube of 10 mm internal diameter filled with KOH solution. A tap was connected to the U-tube in order to set the solution level with atmospheric pressure after the removal of CO_2. The U-tube had two ports, one port for the injection of biogas, and the other port for gas outlet after CO_2 removal. The percentage of CH_4 was measured using KOH solution. The released gas was fractioned in percentage of CO_2 and CH_4 by using 40% KOH solution, **Abdel-Hadi, 2008**. All measurements were performed at room temperature and atmospheric pressure. According to **Hansen, et al., 2004**, values of gas volume were corrected for standard temperature and pressure (STP).
Water displacement method, to estimate the volume of the produced CH_4, the produced gas was passed through 1M NaOH solution contained in an airtight washing bottle in order to remove CO_2. After that, the remaining CH_4 pass into a 500-ml glass container and displaced the water which overflowed into a volumetric cylinder. Volume of the displaced colored water represented the methane volume.
Gas chromatography (GC), GC Model SHIMADZU (Japan) was used to determine biogas components as byproducts of anaerobic digestion process.

2.3 Experimental Procedure
The experimental work was achieved according to the following steps:
Physical pretreatment of GR, The 5-cm length pieces of reed stacks were grounded by using electrical household grinder. The grounded GR particles were sieved using mechanical sieve shaker to prepare size range of 0.3-0.6 mm

Chemical pretreatment of GR, 20 g sodium hydroxide grains were dissolved in 500-ml distilled water to prepare 4% (w/v) NaOH solution. Then the prepared solution was added to 30 g of grounded reed with continuous manual stirring of the mixture using a glass rode. The resulted slurry was placed in the oven overnight at 105 °C, and then the dried alkali-pretreated GR was repeatedly washed with water to remove any excess NaOH.

Inoculum preparation, Chicken dung is known to be rich in the methanogenic anaerobic bacteria. Therefore, it was selected to alternatively inoculate the anaerobic digesters. It was prepared in distilled water as slurry, and then added to the digesters as a supplementary material for enrichment of bacterial activity and hence enhancement the anaerobic co-digestion process.

System setup and start-up of digesters, lab-scale digesters were set up and operated in batch mode to estimate the rate of biogas production from alkali-free and alkali-pretreated GR. The system mainly composed of 500-mL Pyrex borosilicate heatproof code glass bottles setup as the anaerobic digesters. The contents of each digester were maintained at the ratio of 1:10. This ratio is equivalent to 40 g solid waste: 400 ml inoculum slurry. Every single digester was tightly plugged using a rubber stopper contained 2 holes; each of 4 mm diameter through which a small portion of glass tube was submersed into the digester. The other end of the glass tube was connected with rubber tube to transfer the generated biogas to the gas measuring apparatus. In order to prevent the release of produced gas, the rubber stoppers were tightly wrapped with parafilm. Flushing with nitrogen was performed for 10 min to keep the digesters in an anaerobic environment condition. Digesters were placed in a thermostatic water bath to keep them at the required temperature conditions. Manual shaking of digesters was daily performed to allow mixing of the co-substrates (GR and inoculum). **Fig. 1** illustrates the digesters arrangement and set up. **Table 3** presents the digesters contents and conditions.

Residual digestate as a soil amendment, upon the completion of the co-digestion of GR, a residual digestate was resulted as a byproduct of the co-digestion process. Accordingly, in order to examine the feasibility and overall efficiency of this sustainable approach, a decision was made to investigate the validity of utilizing this digestate for soil amendment. Sun flower seeds were selected for this test. The seeds were planted in 2 identical pots, fertilized with the digestate as follows:
Pot (1) Alkali- free GR
Pot (2) Alkali-pretreated GR

3. RESULTS AND DISCUSSION

3.1 Biogas Production

The effects chemical pretreatment of GR and temperature conditions addition on biogas production and methane yield from GR by anaerobic co-digestion process are presented as follows:

3.1.1 The influence of chemical treatment

The breakdown and hydrolysis of cellulose and hemicellulose are difficult and slow, in particular with the presence of lignin. Alkali pretreatment will enhance the destruction of the rigid lignin bonds. **Fig. 2** illustrates the effect of alkali-pretreatment on biogas production and methane yield. The increase in biogas production associated with alkali-pretreatment was due to the destruction of the crystalline structure of lignocellulose by and removal of the surface layer of lignin and hemicellulose. **Kong, et al., 1992** reported that the addition of alkali causes the lignocellulose swelling and partial solubilisation of lignin. The results of this study are in a good agreement with previously reported studies. **Liew, et al., 2011** studied the pretreatment of fallen leaves using 3.5% NaOH, and proved that the methane yield increased by 20% during batch tests. Results reported by **He, et al., 2008** demonstrated a significant increase in biogas recovery using rice straw pretreated with 6% NaOH for 3 weeks at ambient temperature in batch tests.

3.1.2 The influence of temperature

The results revealed higher rate of biogas production and methane yield at thermophilic conditions compared to mesophilic conditions as given in **Figs. 3** and **4** for alkali-free GR and alkali-pretreated GR, respectively. It is well observed that at thermophilic conditions, biogas generated exceeded its generation at mesophilic conditions. This observation could be attributed to the fact that at higher temperature, the hydrolysis of cellulose was boosted resulted in a rapid rate of co-digestion process. These results were in a good agreement with the previously reported studies including but not limited to the study carried out by **Yan, et al., 2013** who suggested that at mild thermal pretreatment (50–120°C), the concentration of soluble organic matters increased gradually with temperature indicating higher rate of hydrolysis.

3.2 Kinetic Model

For anaerobic digester operating in a batch mode, the rate of biogas generation corresponds to specific growth rate of methanogenic bacteria in this digester. Accordingly, the predicted rate of biogas production can be calculated using the ***Modified Gompertz Model*** **Nopharatana, et al., 2007**. This model is represented by equ. (5). A non-linear regression can be used to fit the data **Zwietering, et al., 1990**.

$$G_{(t)}= G_0.\exp\{- \exp [((R_{max}.e)/G_0) (\lambda-t) +1]\} \quad (5)$$

Where:

$G_{(t)}$ = the cumulative biogas yield at a digestion time (mL/g VS)

G_0 = the biogas potential of the substrate (mL/g VS)

R_{max} = maximum methane production rate (mL/g VS.d)

λ = lag phase (day)

t = time (day)

e = exp (1) = 2.7183

In this study, a nonlinear least-square regression analysis was applied using SPSS [IBM SPSS statistics 24 (2009)] to find out λ, R_{max}, and to predict biogas and methane yields. **Figs. 5- 8** illustrate the degree of compatibility between the measured and predicted values. Also, **Table 4** presents the results and kinetic constants obtained by the ***Modified Gompertz Model.*** The well-fitting between the measured and predicted values of biogas recovery was in a good agreement with the previously reported studies. **Matheri, et al., 2015,** suggested that well-fitting was observed for the predicted results estimated by the Modified Gompertz Model with the measured values of biogas recovery from co-digestion of cow manure and grass clippings. **Kafle, et al., 2013,** proved that the measured values of biogas generated from the digestion of fish were in a good agreement with the predicted values computed by Modified Gompertz Model

3.3 Soil Fertilization with Residual Digestate

The results of this part of work revealed that the selected process is a fair sanctioned approach to treat the residues from the co-digestion process of GR. **Fig. 9** presents the growth progress of sun flower seeds after 3 weeks observation period. As shown in this photo, healthy favorable growth of fertilized crop was observed. An additional benefit of this sustainable environmentally friendly approach is the volume reduction of this lignocellulosic material as a result of the co-digestion process.

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Table 1. Chemical reagents details.

Chemical Reagent	Chemical Formula	Purity%	Provider	Purpose of Use
Sodium bicarbonate	$NaHCO_3$	99	BDH, England	pH adjustment
Phenolphthalein	$C_{14}H_{14}N_3NaO_3S$	99	BDH, England	To color the water in the displacement bottle
Sodium hydroxide	NaOH	98	BDH, England	1- Pretreatment of GR 2- For Kjedahl analysis
Potassium hydroxide	KOH	98	BDH, England	CO_2 removal
Hydrochloric acid	HCL	98	BDH, England	For Kjedahl analysis

Table 2. Values of total solids, volatile solids, nitrogen, and carbon contents in the co-substrates before and after the co-digestion process.

Digesters	%TS	%VS	%C	%N	C (g)	N (g)	C/N
Alkali-free GR before digestion	90	95	1.011	5.077	53.33	364.81	0.146
Alkali-free GR after digestion	85	9.60	0.056	0.426	24.68	225.28	0.110
Alkali-pretreated GR before digestion	83	177.75	1.031	4.901	53.93	359.53	0.150
Alkali-pretreated GR after digestion	80	4.28	0.075	0.336	25.25	222.58	0.113

Table 3. Digesters arrangements, contents, and conditions.

Digester #	Digester contents	Temperature Condition
1	Alkali-free GR with chicken dung inoculum	Mesophilic
2	Alkali- pretreated GR with chicken dung inoculum	
3	Alkali-free GR with chicken dung inoculum	Thermophilic
4	Alkali- pretreated GR with chicken dung inoculum	

Table 4. Results of the kinetic study using Gompertz model at mesophilic conditions.

Digesters #	$G_{(t)}$ experimental (mL CH_4/g VS)	Gompertz model parameters				R^2
		λ (day)	$R_{max.}$ (ml CH_4/g VS)	G_0 (ml CH_4/g VS)	$G_{(t)}$ predicted (ml CH_4/g VS)	
1	40.01	8.72	0.31	40.01	33.77	0.98
2	56.76	1.00	0.90	56.76	56.51	0.99
3	38.81	0.40	0.61	38.81	35.50	0.98
4	114.75	1.31	1.93	114.75	108.15	0.99

Figure. 1 Digesters arrangement and set up.

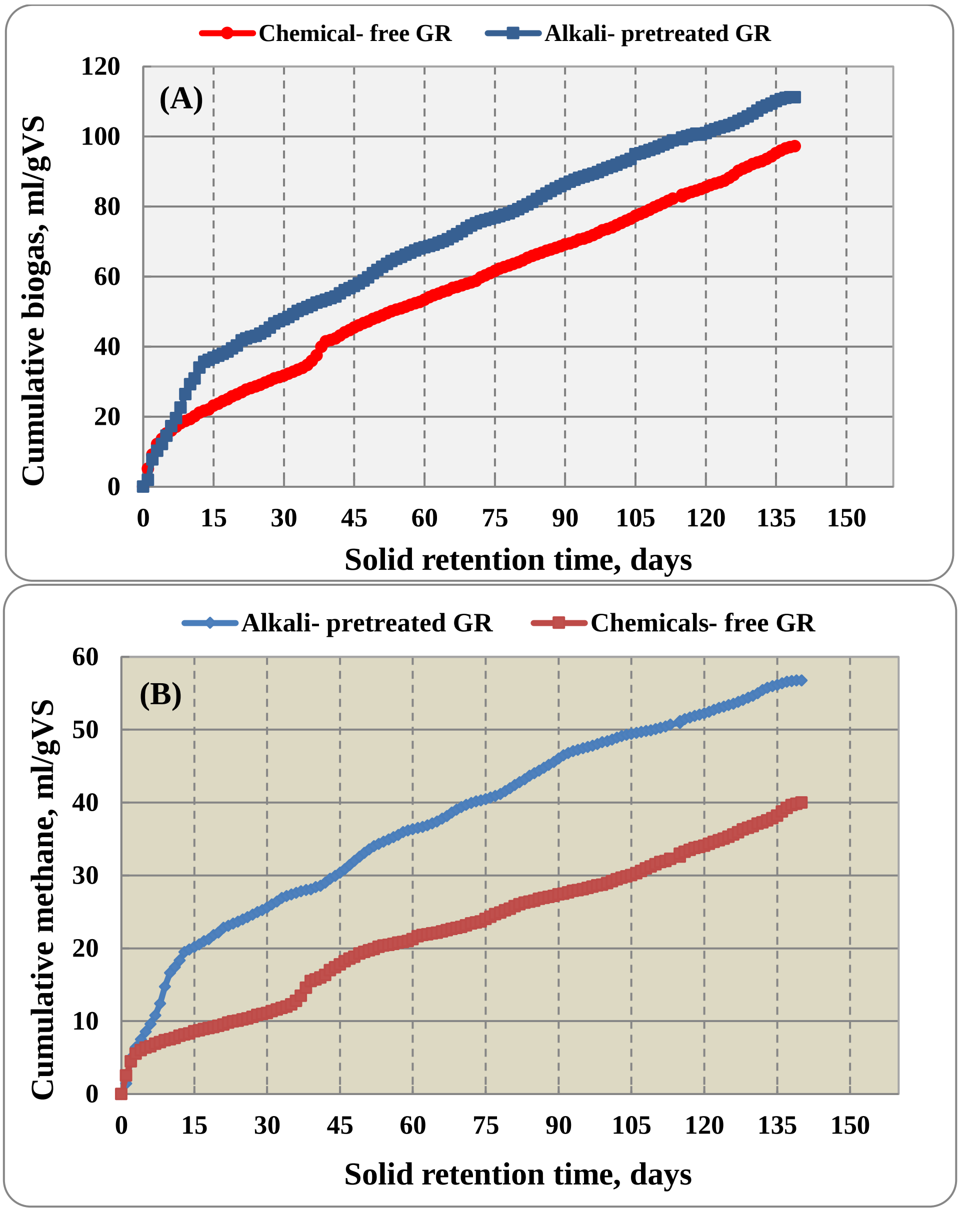
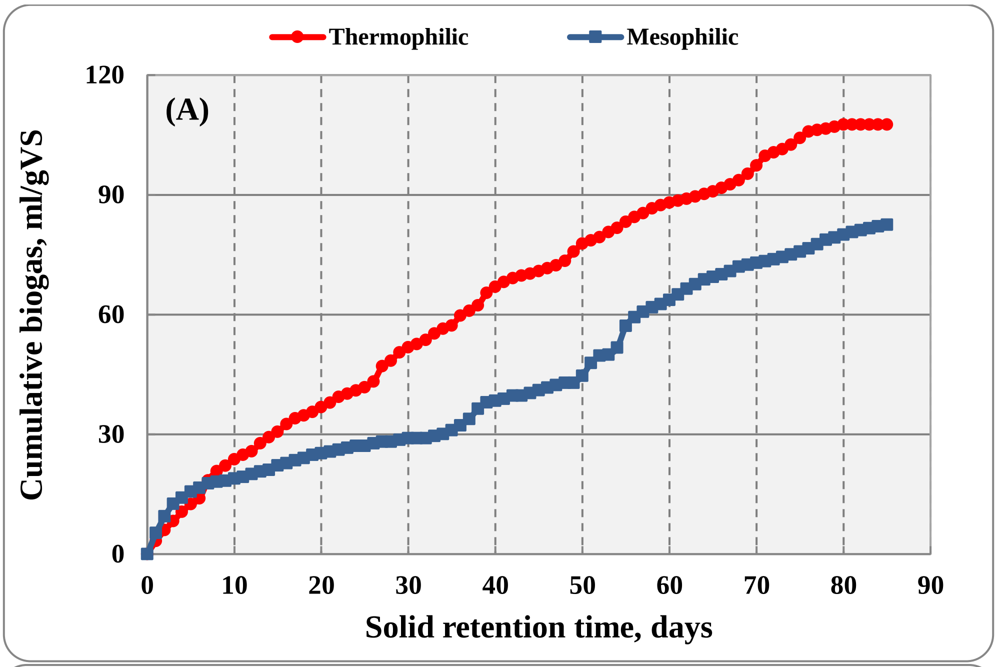


Figure 2. Effect of alkali-pretreatment on the profiles of cumulative, **(A)** biogas production; (B) methane yield from anaerobic co-digestion of GR.



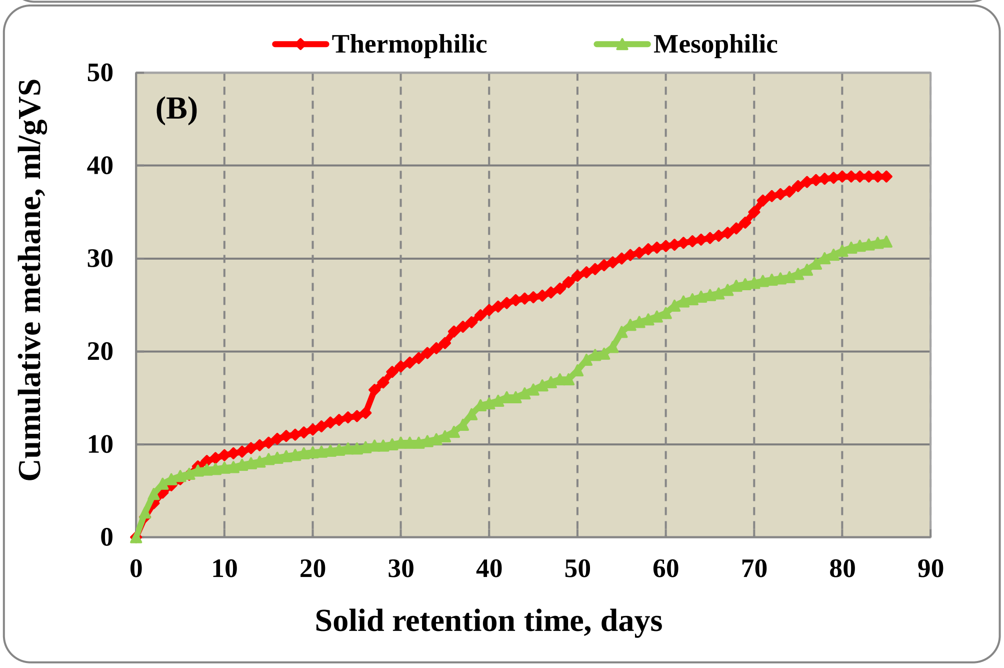


Figure 3. Effect of temperature conditions on profiles of cumulative, (A) gas production; (B) methane yield from alkali-free GR.

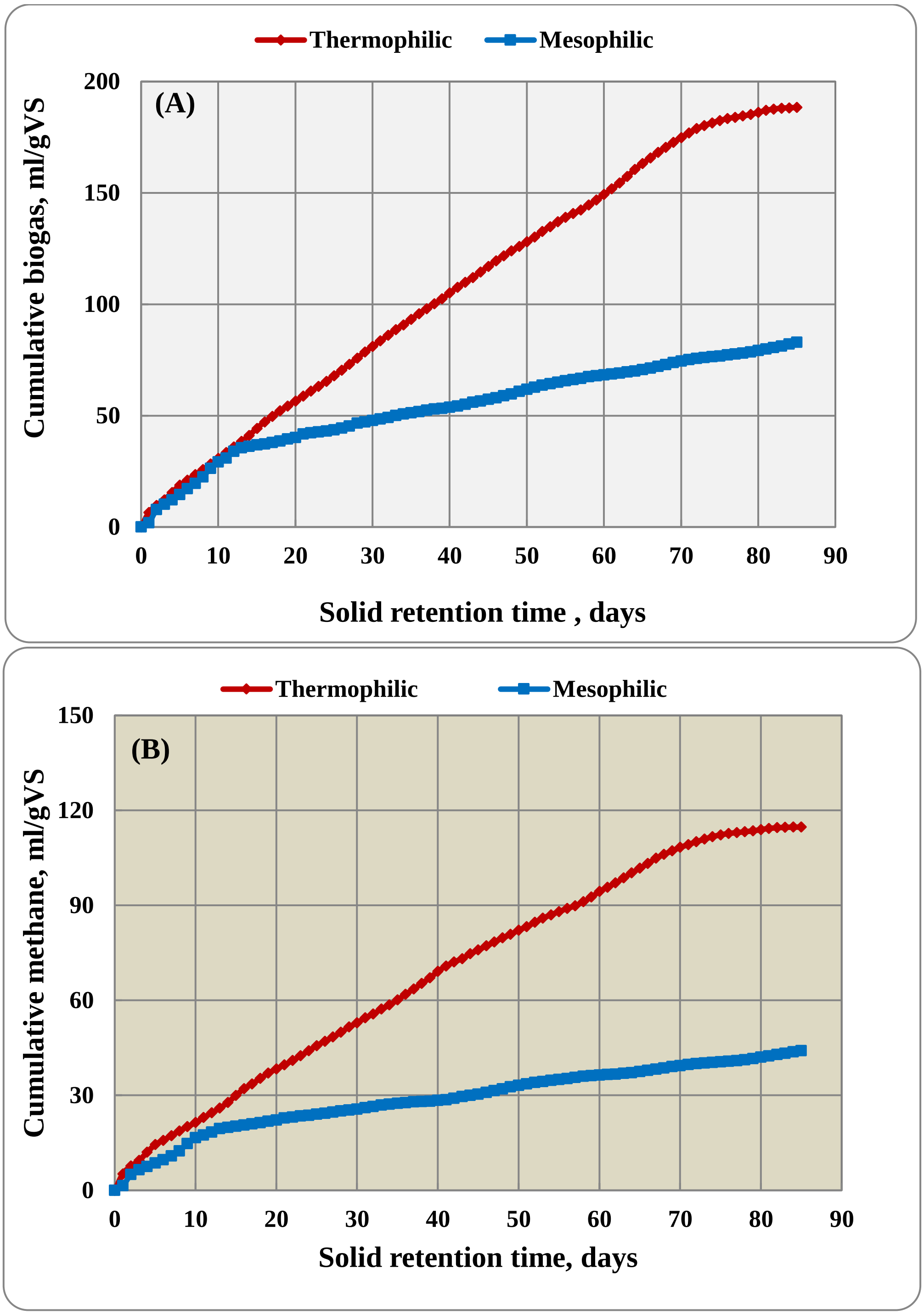


Figure 4. Effect of temperature conditions on profiles of cumulative, (A) gas production; and (B) methane yield from alkali-pretreated GR.

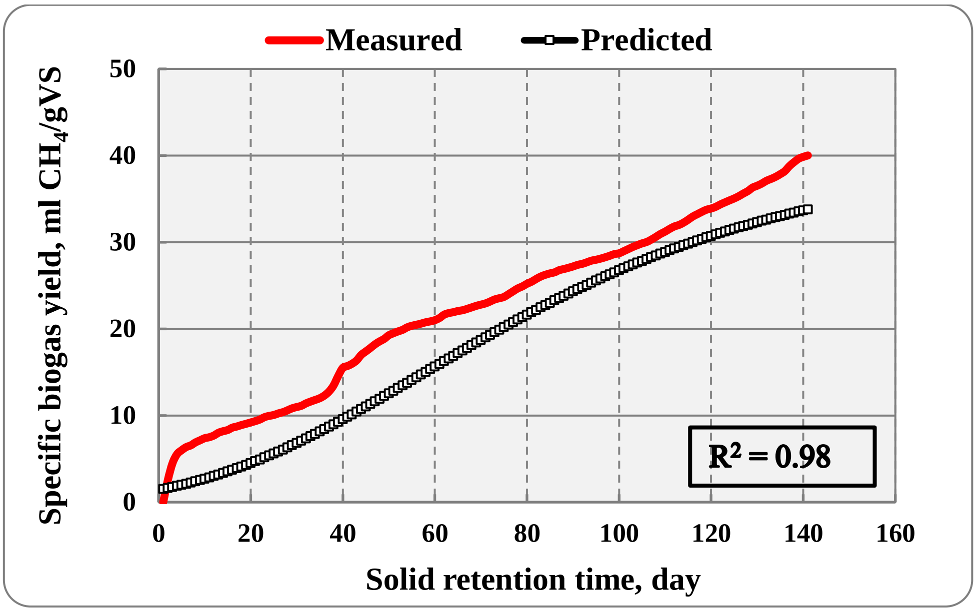


Figure 5. Measured and predicted data for digester 1.

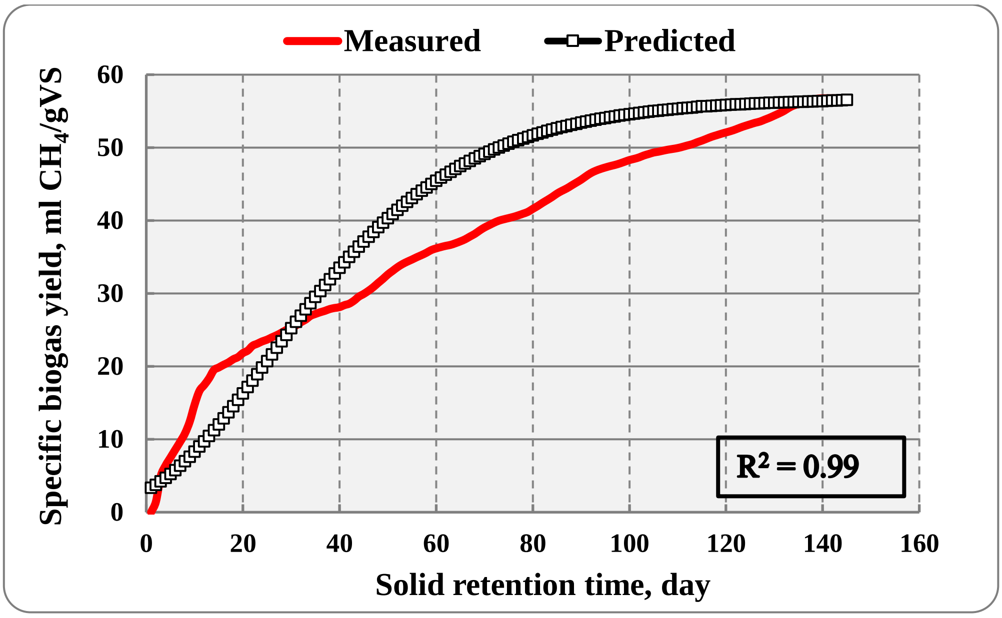


Figure 6. Measured and predicted data for digester 2.

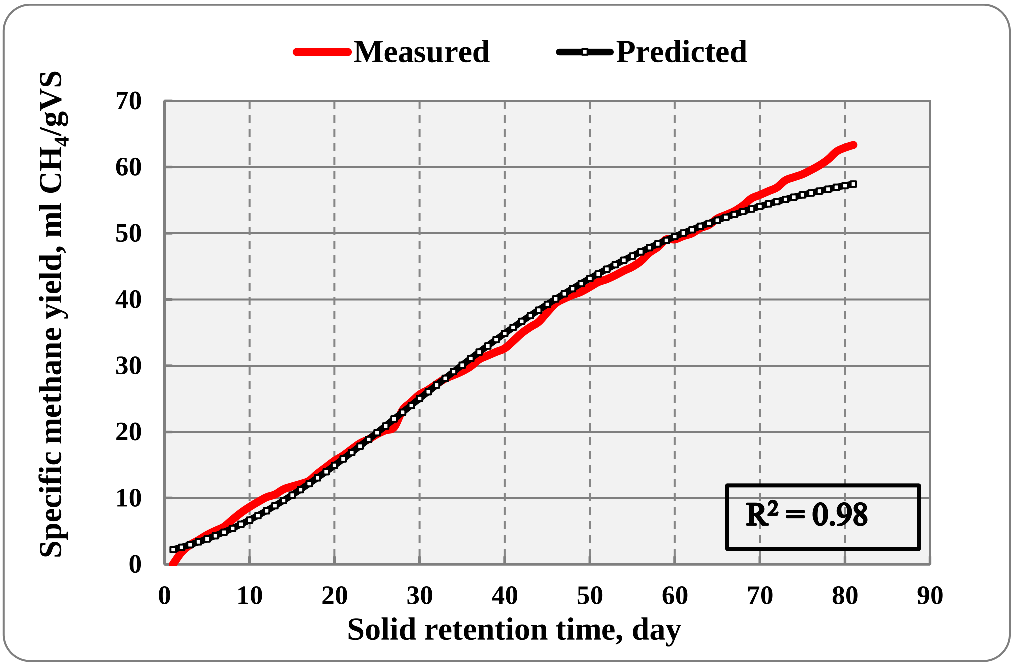


Figure 7. Measured and predicted data for digester 3.

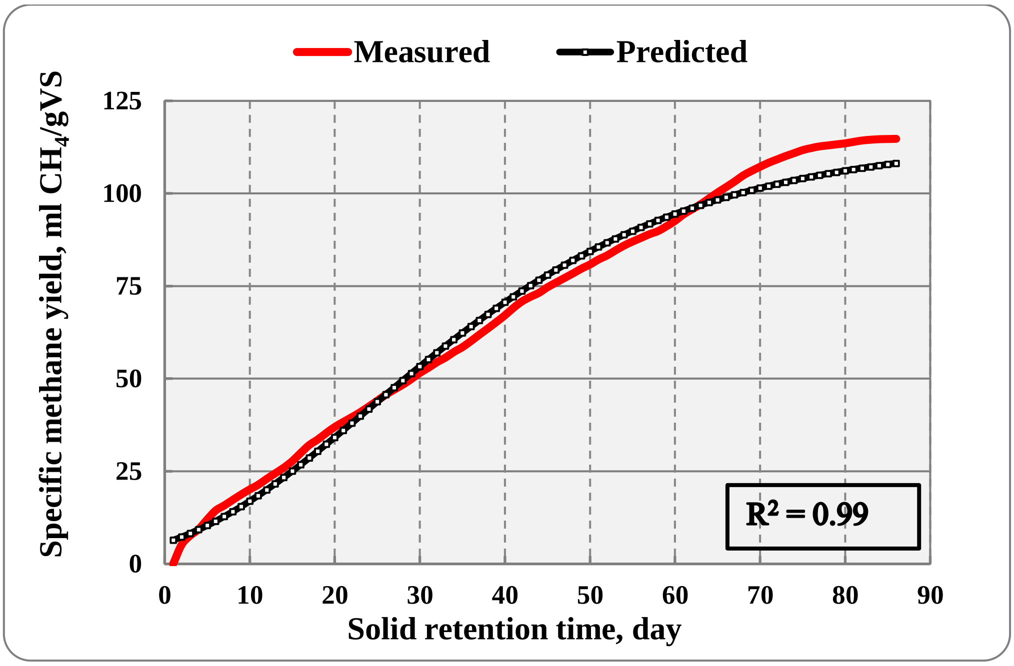


Figure 8. Measured and predicted data for digester 4.

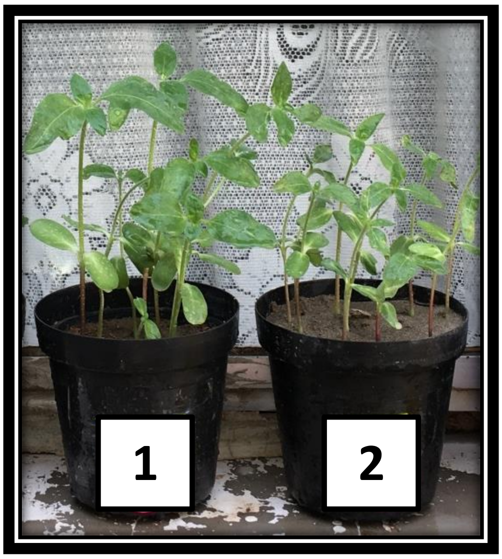


Figure 9. The growth of sun flower seeds after 3 weeks observation period; Pot 1 for Pot 4 for alkali-free GR, Pot 2 for alkali-pretreated GR.